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Original Research Article

Developing New Protocol for DNA Isolation Using House Hold Things

Ms. Deepa K P

Assistant Professor, Department of Botany, Bishop Heber College *(Autonomous)*, Tiruchirappalli -620017

ABSTRACT

In this new era of biotechnological world, genetic knowledge is a must to the need and upto the molecular level study has been implemented in the school subjects itself. The DNA isolation and its structural study is an interesting topic to all levels of learning. Molecular study of DNA is available in Higher Education Institutions and it will be new to the young school students. In this context the objective of my study is to develop a new protocol for DNA isolation. The study has conducted to isolate DNA from 6 tropical fruits and the chemicals used were soap oil and ice- cold alcohol. As bromelain has proteinase activity, pine apple is used to serve the purpose of protein digestion. The DNA clumps were observed in 3 fruits clearly, i.e. Strawberry, kiwi, apple and the other fruits like guava, banana has shown little protein impurity. But in grapes, we are unable to isolate the DNA. This study provides new dimension in developing a protocols for DNA isolation using cheap household things and will be more useful for budding students.

Keywords: DNA isolation, Bromelain, UV- Spectrophotometer, Methylene Blue, Microscope.

INTRODUCTION

Genetic material which defines the entire features of a living object is called as DNA, the deoxyribonucleic acid. DNA is also known as the carrier of genetic information. DNA, a genetic code that replicates itself, exists in all the living objects which are the key constituent of chromosomes. Almost every cell in a body of a person will have same DNA. DNA is majorly situated in the nucleus of the cells which is called as nuclear DNA. Mitochondria will accommodate a minor amount of DNA that is known as mitochondrial DNA, mtDNA or mDNA. DNA can replicate, or make copies of itself which is the key property of DNA. Every element of DNA presents in the double helix could function as the pattern to replicate the bases sequence. As all the newly formed cells necessitate to include replica of the DNA which was there in the mother cells, it would be important when cell splits. There are two types of genetic materials present which are RNA (ribonucleic acid) and DNA (deoxyribonucleic acid). Most organisms have DNA as their genetic material, but few viruses are having RNA. DNA is the genetic material presents in the cytoplasm of prokaryotic cells (bacteria) and the true nucleated cell of plant and animals that regulates the structure of the organisms. Nucleus of each cell has DNA which would be exactly same in each and every cells. RNA as well as DNA naturally forms double helices during the presence of its inverse complement (the order which has suitable bases for forming double helix).

Chromosomes have thread like structures that are present in the nucleus of the cell of animals and plants. They are made of protein and one molecule of deoxyribonucleic acid (DNA). Throughout the division of cells, the genetic materials were tightly packed and remain uniformity all over the cells. (Sam Brooks et al. 1989).

Every cell of human typically comprises twenty-three couples of chromosomes and a total of forty-six. 22 of these couples known as autosomes, looks similar in females and males. The twenty third pair is the sex chromosome which differs for females and males. The largest chromosome human is Chromosome 1 which spans approximately 249 millions of DNA building blocks and represents about 8 % of the over-all DNA of the cells. The chromosome 21 is both tiniest human auto some and chromosome, which has forty eight millions of nucleotides, denoting approximately 1.5 % of the over-all DNA of the cells. Chromatids would be divided during metaphase to replicate a cell during mitosis. Dependent on the chromosome, they have specific genes coded into their DNA make up, that can be expressed in the individual, or lie dormant until a certain event triggers them. A chromosome that contrasts from a normal autosome in terms of behavior, size and formis known as allosome. Adistinctive pair of allosomes that determines the sex of a baby formed via sexual reproduction is the human sex chromosome. Autosome appears in twosomes (pair) whose members have the same form but diverge from other pairs in a diploid cell, whereas members of an allosome pair might diverge between one and the other. Hence it determines the sex. Nucleosideis aglycosylamine that contains one nucleobase and afive carbon sugar (deoxyribose or ribose) whereas a nucleotide contains a nucleobase, a five-carbon sugar and one or more phosphate group. Base of nucleosideis bound to deoxyribose or ribose through a link of beta-glycosidic. Few examples of nucleosides are given below:
Inosine, thymidine, guanosine, adenosine, uridine, andcytidine. (Dellaportaet al 2012)

Structure of DNA

The structure of DNA is a double-helix made with base pairs fixed to a back-bone of sugar phosphate. Nucleotides are organized in a couple of long elements that forms a spiral known as double-helix. Arrangement of this double-helix is almost similar to the structure of a spiral ladder. Base-pairs make the steps of this ladder. The molecules of sugar and phosphate make the vertical side-rails of the ladder. Every nucleotide has phosphate and sugar particle that form the backbone of DNA using one of the 4 bases. These organic bases are listed below:

- Thymine (T)
- Cytosine (C)
- Guanine (G)
- Adenine (A)

The bases of DNA pairs with one another i.e., Cand G, A and T to make components named base-pairs. Each base is attached to one sugar and one phosphate molecules. One base sugar and one phosphate are together known as nucleotides.

MATERIAL AND METHODOLOGY

Materials:

- 5 Fruits- Apple, Banana, Guava, Grapes, Kiwi, Straw berry
- Funnel
- Measuring spoon, salt crystals soap oil and a bowl.
- Cheese-cloth
- Tall tumbler (glass)
- small glass vessel/pot and re-sealable plastic bag

Utensils

Micropipette, Beaker - 250 ml, Measuring jar, Funnel, Test tubes, Culture tubes, Eppendrop

Centrifuge tubes, Ethanol- ice cold, Salt solution, Soap oil, Cheese cloth, Tooth pick, Glass rod

Ethanol has to be chilled with the help of freezer and this has to be filled in the tall tumbler/glass. Mix 1 ½ spoons of crystal-salt, ½ cup of clean-water and 1 spoon of soap oil in a cup/bowl. Keep this solution (called as extraction liquid) on the side for few minutes to allow reaction. Then cover the funnel fully using cheese-cloth. Put this funnel-tube into the tall tumbler/glass (Tumbler which contains the extraction liquid). Wash/clean and peal the fruits, and extract the pulp from the fruits.

Procedure

Keep the pulp of these fruits into a resealable plastic bag and then remove the extra air from the bag. Then seal this bag with your fingers. Crush/squeeze the fruits (apple, kiwi, guava, banana, grapes, strawberries) in separate packet smash for three Mins. Mix two table-spoons of the solution (extraction liquid /soap oil) with the fruit smash that was kept in the plastic bag. Then remove all the extra air and re-seal the plastic bag again. Mix two spoons / 5 ml of pineapple pulp. Give 5 minutes time for reaction. Again squeeze the fruit mixture with your fingers. Transfer the processed fruit juice from the tall tumbler to the small glass so that the glass is one quarters full. Add pineapple juice of one tablespoon to each fruit liquid packet. Give 5 minutes time to react. Take one and half cup of chilled ethanol. Title the glass and gradually add the chilled ethanol. Keep adding till the ethanol forms about one inch deep layer on the surface of the fruit juice. You might not be requiring all the one inch layer. Ensure that the fruit juice and ethanol will not mix-up. Examine the solution inside the glass wherein the fruit DNA will be appeared as a goocy, clear, white, stringy material on the top surface.

Quantification of DNA with Spectrophotometer

1x TE solvent suspended DNA was taken and spectrophotometric analysis was done in a quartz cuvette. Zeroed the instrument with solvent as blank. For a path length of 1 centimeter, the optical density in 260nm (i.e., OD-260) matches 1.0 for the solutions given below:

33 µg/ml solution of ssDNA,40 µg/ml solution of RNA 20-30 µg/ml solution of oligonucleotide,50 µg/ml solution of dsDNA. Compute the OD-260 / OD-280 ratio for an indication of purity. An OD-260 /OD-280 of 1.8 would be there for pure DNA, An OD-260/OD-280 ratio of 2.0 would be there for pure RNA. Lower ratios can be produced due to protein or phenol contamination (Carlos et. al 2001)

RESULTS AND DISCUSSION

The DNA isolation using a known protocol is easy to follow. But to prepare a new protocol with cheap household things is much interesting. In this study, we isolated DNA, the genetic material with the help of low budget things daily used in home. The only chemical we are using out of this was ice cold ethanol. But we observed DNA clogs at the end of this study. The Fruits were smashed with soap oil and pineapple juice was added to it as, bromelain act as a proteinase agent. The soap oil having sodium derivatives of palmate and stearate will lyse the cell wall and the cytoplasmic organelle physiologically and physical grinding was done by mechanical pressure. The fruit smash was filtered and then the clear filtrate has been added by ice cold ethanol through the side of the vessel. Effervescence of DNA formed at the place of joining these liquids (Fig: 1).

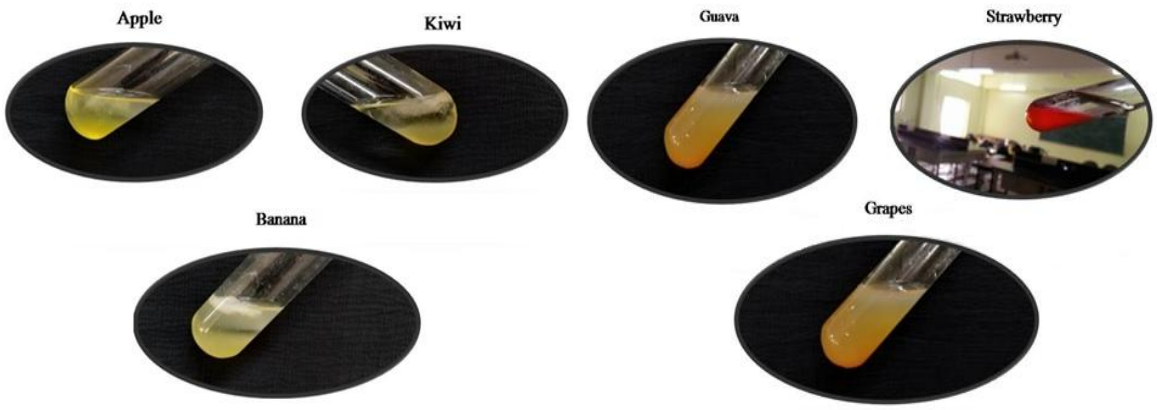


Figure 1. DNA effervescence during ethanol addition

Further to check the purity of genetic material, quantification using spectrophotometry was done. The UV reflected intensities were noticed at a wavelength of 260 nm and 280 nm respectively. The results were tabulated as:

Table 1: Quantitative estimation of DNA in UV spectrum.

Sl. No	Fruits	OD at 260nm	OD at 280 nm	Ratio of OD260/OD280
1	Apple	1.1493	0.716	1.6
2	Banana	1.1395	1.1206	1
3	Guava	1.234	0.8798	1.5
4	Kiwi	1.8338	1.0745	1.7
5.	Strawberry	1.4829	0.8213	1.8

About 6 fruits (Apple, Kiwi, Grapes, Guava, Strawberry and Banana) were taken for the DNA isolation studies. Of which the DNA clump formed during alcohol adding was observed in 5 fruits only. Grapes didn't show any DNA clumps. It may immerse in the alcohol. In the quantitative study, the pure DNA was obtained in Kiwi and Strawberry. Apple shows some quantity near to the pure value. But Banana, Guava is not upto the pure DNA mark level. High protein and carbohydrate content in these fruits may be the reasons behind this. (Puchoo 2011).

Also in the chart analysis it is clearly mentioned the light intensity of DNA samples for UV range at two wavelengths for the five fruits Fig: 2

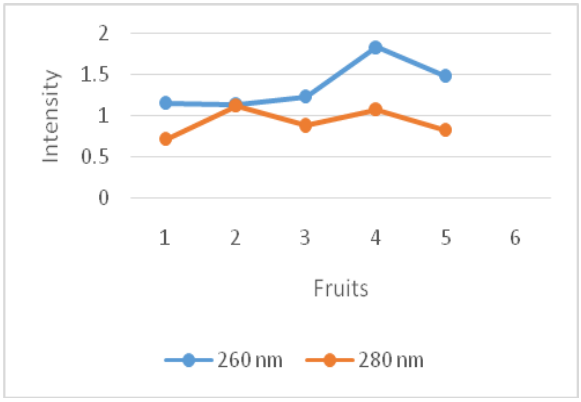
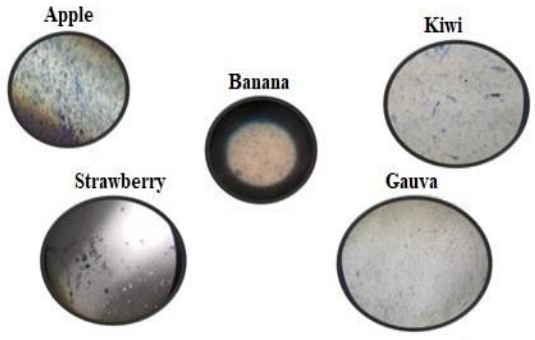


Figure 1.UV spec analysis of DNA clumps from various fruits.

Qualitative study by light microscopy was done and the DNA fragments were stained with 0.1% Methylene Blue stain. Images where taken at 100x magnification and the images were shown in Fig:3. DNA clumps were formed as broken threads and visualized in 100x objective ad images obtained by camera attached through it.



It is very easy to isolate the genetic material in our home itself. By this work it will be more interesting to budding students that the mystery of life and heredity can be

visualized in such a simple way. This study meets our purpose and it will be a simple and easy protocol to isolate the genetic material using house hold things.

CONCLUSION

The results of this study provide a unique insight into the potential applications of technology in education through the attitudes and perspectives of pre-service teacher and students. The findings will assist both present and future educators at all levels to purposefully develop and implement appropriate learning techniques for effective and engaging teaching and learning opportunities.

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